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USE OF A MIXTURE FOR THE PRODUCTION OF AN AGENT FOR TREATING DEFECTIVE OR DEGENERATED CARTILAGE IN VIVO AND FOR THE PRODUCTION OF NATURAL CARTILAGE REPLACEMENT IN VITRO

The invention relates to a method for using a mixture for the production of an agent for the treatment of defective or degenerated cartilage in vivo in accordance with the introductory portion of claim 1, as well as to the use of this mixture for the production of natural cartilage replacement in vitro in accordance with claim 9.

Permanent pain, immobility and an impairment of the joint are typical indications of injury to the cartilage due to an accident or osteoarthrosis. The success of surgical interventions in joint injuries, such as the osteotomy, transplantation of the perichondrium or the use of an arthroplastic material is limited. As a rule, the natural hyalin structure of a healthy cartilage is never attained by surgery.

For treating cartilage defects, every effort is made to implant frameworks of polymer materials, which will be colonized with chondrocytes. These materials function here as carrier material for the chondrocytes and are available as absorbable or not absorbable materials. In recent years, frameworks from natural and synthetic absorbable carrier materials were developed and tested. In so doing, it was noted that cartilage-like constructions, which had been raised in vitro, attained neither the biochemical nor the biomechanical properties of in vivo tissue.

Several methods are used for the clinical treatment of cartilage defects.

In the past, the damaged cartilage tissue was removed predominately mechanically. New treatment methods transplant chondrocytes and periosteum or perichondrium for closing the lesion.

The method of milling out was described for the first time in 1959 by Pridie. The method of abrasive removal was developed in the 1980s. Both methods are based on the same principle. The defective cartilage sites are removed to the bleeding bone. Enough cartilage is removed so that the transition from bone to cartilage is formed exclusively by undamaged cartilage. The healing of the cartilage is promoted by the rich supply of nutrients of the opened blood vessels of the bone. Numeral studies have shown that the regrown tissue consists predominantly of fiber cartilage and not of the hyaline cartilage, which would actually be necessary for a permanent regeneration.

Other methods make use of osteochondral transplants. As autograph or allograph, these transplants are inserted into the cartilage defect and anchored in the subchondral bone. In the first case (autograph), the organ donor and the host are one and the same person and, in the second case (allograph), they are different persons, but from the same species. Cylindrical cartilage studs, together with the subchondral bone, are removed from the donor region with the help of a stamping tool and anchored in the defect zone by means of a prefabricated press fit. One or more studs (➔mosaic plastic), depending on the size of the defect zone, are used to close the damaged surface.

For transplanting chondrocytes, the latter are removed from cartilage regions of the knee, which are not stressed as much. The cells removed are propagated for 14 to 21 days in nutrient solution. After they have been cultured, the cells are injected into the region of the defect and covered with a piece of periosteum or perichondrium. After 2 years, it can be shown by a biopsy that hyaline cartilage has formed. In one study, the clinical result of 14 of 16 patients was described as good to very good. A study in Sweden with 400 patients showed comparable results.

The function of cartilage in joints consists, on the one hand, of absorbing and distributing forces, which arise when the joint is stressed, and, on the other, of making available a lubricating surface, which prevents the abrasion and degradation of the joint. The first function is ensured by a unique composition and structure of the extracellular matrix. On the other hand, the second function depends on a functional cartilage-synovial fluid interface. There is interference with these functions especially in patients with cartilage surfaces, which are degeneratively changed or otherwise affected.

The invention is to provide a remedy here. It is an object of the invention, on the one hand, to provide an agent for the treatment of defective or degenerated cartilage in vivo and, on the other, to make available an improved production of natural cartilage replacement in vitro, especially for cartilage defects in the joint region.

Pursuant to the invention, this objective is accomplished with an agent, which has the distinguishing features of claim 1, as well as with a use of this agent, which has the distinguishing features of claim 9.

As lubricin, the lubricating glycoprotein-1 (LGP-1) is named, which is produced from the same gene as the megakaryocyte stimulating factor (MSF) by alternative splicing. Lubricin has a molecular weight of approximately 230 kDa (purified form in human synovial fluid) and is glycosylated to a high degree.

As proteoglycan 4 (PRG4), the surface zone protein (SZP) is named, which is obtained by alternative splicing from the MSF gene. It has a molecular weight of approximately 340 kDa (from human joint cartilage) and carries several oligosaccharides groups, as well as glycosaminoglycan chains. It has turned out that the use of SZP and similar substances (group A) in the inventive mixture not only has a strong lubricating effect, but also acts as a

chondro-protective molecule, which gives protection for the lower-lying cartilage cells.

Originally, SZP was isolated and purified from culture liquids from explants, which originated from the surface zone of bovine cartilage. SCP can be synthesized by chondrocytes in the surface zone, but not by those from the middle and lower zones.

Hyaluronic acid consists of glucouronic acid and acetylglucosamine, which build up the disaccharides, hyalubironic acid. As a result of its filamentous, unbranched molecular structure, hyaluronic acid forms highly viscous solutions. Admittedly, hyaluronic acid does not have any direct lubricating properties. However, it is important for the rheological behavior of the synovial fluid by adjusting the viscosity suitably. Such an adjustment prevents the synovial fluid flowing out during the loading phase of the joint.

Surprisingly, it was found that the mixing of lubricin (or similar substances of group A) with a hyaluronic acid (or similar substances of group B) in a suitable solvent reinforces the action of these two substances in a synergistic manner.

Further advantageous developments of the invention are characterized in the dependent claims.

The advantages, attained by the invention, are, essentially, the following:

- In patients with osteoarthrosis, the improved lubrication results in a reduction in pain and in a delay or even complete prevention of further degradation of the cartilage.
- In patients with hemiarathroplasty, a reduction in the cartilage degeneration and an improved abrasion of the artificial joint can be expected because of

the improved lubrication. As a result, the service life of the implant increases and a revision can be prevented or delayed.

- In patients with cartilage trauma or surgical interventions, the shear forces at the wound are reduced due to the lubrication. As a result, there is better healing of the two halves of the tissue.
- The lubrication of joints in the case of osteoarthrosis, hemiprostheses, after an osteochondral transplant and autologous cell transplant (ACT) or after a meniscus operation.

The improved lubrication is achieved with natural joints (especially in cases of osteoarthritis and rheumatoid arthritis) as well as with artificial joints. In cases of a total of hip prosthesis, the lubrication between the polyethylene of the acetabulum component and the metal of the hip head of the shaft component is improved.

In the case of a special embodiment, the phospholipids used are surface active in nature. The interfacial lubrication, resulting therefrom, is responsible for less cartilage damage in the further course.

Advisably, the hyaluronic acid used has a molecular weight of at least 1×10^6 Da.

Advantageously, the ratio by weight of the substances of group A (lubricin, proteoglycan 4 (PRG4) and phospholipids (SAPL)) to the substances of group B (hyaluronic acid, glycosaminoglycan and derivatives of these substances) ranges from 0.05 to 0.40 and preferably from 0.08 to 0.25.

The solvent, which is to be used, advantageously is a Ringer solution, preferably a physiological salt solution.

The concentration of substances of group A in the solvent preferably ranges from 0.02 to 0.05% by weight and that of substances of group B preferably ranges from 0.2 to 0.4% by weight.

The mixture of one or more substances of group
A) lubricin, proteoglycan 4 (PRG4) and phospholipids (SAPL)
with one or more substances of group
B) hyaluronic acid, glycosaminoglycan and derivatives of these substances
dissolved in a solvent,
can also be used for the production of natural cartilage replacement in vitro. Such a mixture can also be used for a method of producing a cartilage replacement material for cartilage defects in the joint region, an open-pored, elastic cell carrier body being populated in its pores with chondrocytes and this mixture, dissolved in a physiologically acceptable solvent, being brought into contact with the chondrocytes.

For this method, the solvent preferably is moved with a lamina flow over the cell carrier body.

In the case of a particular embodiment of this inventive method, an axial force and a rotational force are applied on the cell carrier body simultaneously with a ball a joint-like device. Preferably, the rotation of the ball joint-like device is carried out about two axes, which are orthogonal to one another. The advantage of this measure is based therein that, with an appropriate phase shift, movement trajectories can be set, which come close to those of human joints with respect to distance, form and speed.

The invention and further developments of the invention are explained in even greater detail in the following by means of several examples.

Example 1

Lubricin (4 mg) and 40 mg of hyaluronic acid were dissolved in 20 mL of physiological salt solution (Ringer solution). Over a period of 10 weeks, 2 mL of the solution, so obtained, were injected in situ once a week into the knee joint of a patient with osteoarthritis. Before the injection, the joint was aspirated, in order to prevent dilution of the solution injected.

The patient treated therewith had less pain and improved mobility of the knee joint. A further flushing at a later time showed a distinct reduction in loose cartilage particles in the aspirate.

Example 2

Lubricin (4 mg) and 40 mg of glycosaminoglycan were dissolved in 20 mL of physiological salt solution (Ringer solution). For a period of 10 weeks, a volume of 1 mL of the solution, so obtained, was injected in situ once a week into the hip joint of a patient with osteoarthritis. Before the injection, the joint was aspirated in order to prevent dilution of the solution injected. The patient treated therewith had less pain and improved mobility of the hip joint.

Example 3

Lubricin (5 mg) and 40 mg of hyaluronic acid dissolved in 20 mL of physiological salt solution (Ringer solution). For a period of 5 weeks, a volume of 2 mL of the solution, so obtained, was injected in situ once a week into the finger joints of a patient with rheumatoid arthritis. Before the injection, the joint was aspirated in order to prevent dilution of the solution injected. The patient treated therewith had less pain and a better function of the hand due to the increased extent of movement of the finger joints.

Example 4

After an osteochondral transplantation, a solution of 6 mg lubricin and 45 mg hyaluronic acid was injected into the closed joint capsule of a patient. The solvent consisted of 25 mL of physiological salt solution (Ringer solution), into which 5% of human serum of the same patient had been mixed. The endoscopic examination after the physiotherapeutic therapy of the joint showed improved healing of the sections between the host and donor tissue. The patient was free of pain and could undertake his usual activities.

Example 5

Chondrocytes were isolated from the region of the surface of the knee joint, which carried no weight and had a defect and implanted directly into an open-pored elastic cell carrier body. The cell carrier body consisted of a cylindrical, porous, biodegradable polyurethane framework having a size of 8 mm x 4 mm, corresponding to that of the defect. The cell density was 25-30 x 10^6. The cell-carrier body, the pores of which were populated with chondrocytes, was cultured in "Dulbecco's modified Eagles medium" (DMEM), to which 5% of human serum (of the same patient), a number of nonessential amino acids, namely l-alanine (0.89 mg/L), l-asparagine (1.32 mg/L), l-aspartic acid (1.33 mg/L), l-glutamic acid (1.47 mg/L), glycine (0.75 mg/L), l-proline (1.15 mg/L) and l-serine (1.05 mg/L), as well as 40 μg/L l-proline had been added.

Two days after the cell carrier had been populated, 50 μg/mL of ascorbic acid were added. In addition, immediately before the start of the mechanical stress, 0.2 mg of lubricin and 2 mg of hyaluronan per milliliter of medium where added, of which 3 mL were required. The medium was exchanged daily. After a six-day cell culture, the cell carrier body was subjected to the mechanical stress as described below.

The mechanical stressing of the cell-carrier body took place in a so-called bioreactors system, in which the cell-carrier body was subjected to the action of a ball, so that rotational as well as axial forces could be exerted on the

cell-carrier body. Twice a day, a one-hour mechanical stress of this type was exerted on the cell-carrier body. In one series of experiments, this procedure was carried out from 3 days after 28 days.

The aforementioned addition of 0.2 mg of lubricin and 2 mg of hyaluronan resulted in an improved production of functional cartilage-like tissue, which, after implantation in a cartilage defect, showed an improved physiological effect and led to optimum healing of the cartilage defect.